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Investigation of displacer equilibrium properties and mobile phase operating conditions in ion-exchange displacement chromatography

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Abstract

One of the major advantages of displacement chromatography is the simultaneous concentration and purification that can be effected during the process. The steric mass-action model of non-linear ion-exchange chromatography is employed to investigate the affects of mobile phase salt concentration and displacer equilibrium properties on the concentration of proteins in isotachic displacement zones. The results indicate that the salt microenvironment in displacement zones plays a major role in determining the concentration of the displaced proteins. The parameters which affect the salt microenvironment are both the initial salt concentration in the carrier as well as the induced gradient produced during the displacement process. For a given breakthrough volume, the induced gradient is determined by the ratio of the steric factor to the characteristic charge of the displacer. These results indicate that the use of relatively low salt concentrations in the carrier along with displacers with relatively high steric factor to characteristic charge ratios will produce significant concentration of the proteins during the purification process.

1. Introduction

Ion-exchange displacement chromatography provides an attractive method for overcoming the deleterious effects of peak tailing encountered in conventional overloaded and gradient elution chromatography of proteins. Separations performed in the displacement mode offer the ability to both concentrate and purify solutes in a single chromatographic operation. In addition, under appropriate conditions displacement chromatography can offer an improvement in the selectivity achieved under conventional elution modes of chromatography. While these advantages are clearly well suited for the purification of biopharmaceuticals, the displacement mode of chromatography is seldom considered as a viable option in the pharmaceutical and biotechnology industries. A major reason for this inertia is the lack of understanding of simple cause and effect relationships between the choice of various displacers and chromatographic operating conditions and the outcome of displacement separations.

Operationally, displacement chromatography is performed in a manner similar to elution chromatography where the column is subjected

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to sequential step changes in the inlet conditions. In ion-exchange displacement chromatography the column is initially equilibrated with a carrier solution at constant pH and ionic strength. The feed mixture is then introduced onto the column and immediately followed by a solution containing the displacer. The displacer is chosen such that its affinity for the stationary phase is greater than any of the components in the feed. Under appropriate conditions the displacer forces the desorption of the feed components which develop into adjacent "square wave" zones of homogeneous material. If the column is long enough the system will reach an isotachic state moving at the velocity of the displacer; the least strongly adsorbed feed component appearing first in the displacement train.

Experimentally, ion-exchange displacement chromatography has been employed for the purification of proteins by a number of researchers [1–8]. To date, the development of these separations has relied on trial and error experimental procedures and common rules of thumb. In general, when a displacement separation is not fully developed (i.e., the displacement train is not comprised of homogeneous "square wave" zones), a more completely developed separation can be effected by: increasing the length of column, decreasing the amount of feed, or modifying the mobile phase operating conditions to an environment where the feed components are more strongly retained (i.e., lower salt conditions).

These rules of thumbs, however, address the *attainment* of the fully development isotachic displacement train. The steric mass-action (SMA) model of non-linear ion-exchange chromatography [9] is able to accurately predict induced salt gradients and complex behavior in protein displacement systems [6–8]. Equilibrium in the model is characterized by three independent parameters for each solute: the characteristic charge, the equilibrium constant and the steric factor. In order to design effective displacers for protein purification, it is critical to understand what displacer properties are required to give sufficient affinity and to minimize induced salt gradients. An analytical solution developed for ideal ion-exchange displacement chromatography [9] provides a simple and rapid method for addressing this question. In this paper we examine how properties of the displacer as well as the prevailing mobile phase salt conditions effect the *final* isotachic displacement profile.

2. Theoretical background

2.1. SMA ion-exchange equilibrium

The analytical solution to ideal ion-exchange displacement chromatography based on the SMA ion-exchange equilibrium formalism has been reported elsewhere [9]. An abbreviated form of the ideal SMA displacement model is presented here.

Consider an ion-exchange resin with a total capacity, Λ, equilibrated with a carrier solution containing salt counterions. Upon adsorption, the protein interacts with ν_i stationary phase sites, displacing an equal number of monovalent salt counterions. The adsorption of the protein also results in the steric hindrance of σ_i salt counterions. These *sterically* hindered salt counterions are *unavailable* for exchange with the protein in free solution. For a system of n polyelectrolytes and a single mobile phase modifying salt, n expressions can be written to represent the stoichiometric exchange of each individual protein with the salt.

$$C_i + \nu_i \bar{Q}_1 \rightleftharpoons Q_i + \nu_i C_1 \quad i = 2, 3, \ldots, n+1 \qquad (1)$$

where C and Q are the mobile and stationary phase concentrations, and the subscripts i and 1 refer to the protein and salt, respectively. The overbar, $\bar{}$, denotes bound salt counterions *available* for exchange with the protein. The equilibrium constants, K_{1i}, for the ion-exchange process is defined as:

$$K_{1i} \equiv \left(\frac{Q_i}{C_i}\right) \cdot \left(\frac{C_1}{\bar{Q}_1}\right)^{\nu_i} \quad i = 2, 3, \ldots, n+1 \qquad (2)$$

Electroneutrality on the stationary phase requires:

$$\Lambda \equiv \bar{Q}_1 + \sum_{i=2}^{n+1} (\nu_i + \sigma_i) Q_i \tag{3}$$

For a single protein, substituting Eq. 3 into Eq. 2 and rearranging yields the following implicit isotherm.

$$Q_i = K_i C_i \cdot \left(\frac{\Lambda - (\nu_i + \sigma_i) Q_i}{C_1} \right)^{\nu_i} \tag{4}$$

The equilibrium stationary phase concentration of the protein, Q_i, is implicitly defined in terms of its mobile phase concentration and the concentration of the salt. Thus, once the SMA equilibrium parameters of the protein are determined [10], the isotherm of the protein can be constructed at any mobile phase salt condition. In addition, Eqs. 2 and 3 provide $n + 1$ equations which implicitly define the multicomponent equilibrium for n proteins and a single salt counterion.

In ion-exchange displacement chromatography, adsorption of the displacer produces an induced salt gradient which travels ahead of the displacer. This increase in salt concentration is *seen* by the feed components traveling in front of the displacer and results in a depression of the feed component isotherms. Thus, the traditional Tiselian treatment of displacement, using a displacer operating line and single component isotherms, first requires the determination of the local salt microenvironment *seen* by each of the feed components since the initial carrier salt conditions do not apply to the feed components. A schematic of an SMA ion-exchange displacement separation of a binary ($n = 2$) feed mixture under ideal isotachic conditions in depicted in Fig. 1 [9].

The breakthrough volume of the displacer is given by:

$$V_{Bd} = V_f + V_0(1 + \beta\Delta) \tag{5}$$

where the slope of the displacer operating line, $\Delta = Q_d / C_d$, is calculated using the displacer's single-component SMA isotherm.

$$\Delta = \frac{Q_d}{C_d} = K_{1d} \cdot \left(\frac{\nu - (\nu_d + \sigma_d) Q_d}{(C_1)_d} \right)^{\nu_d} \tag{6}$$

and $(C_1)_d$ is the concentration of salt the displacer *sees* (i.e., the carrier salt concentration, C_1^0).

The local concentration of salt in the isotachic zone of pure feed component i, under the induced salt gradient conditions is given by:

$$(C_1)_i = \frac{\Lambda - (C_1^0 + \nu_d C_{fd}) \cdot \dfrac{\Delta}{\Omega}}{\dfrac{1}{\lambda} - \dfrac{\Delta}{\Omega}} \tag{7}$$

and the concentration of feed component i in the zone is given by:

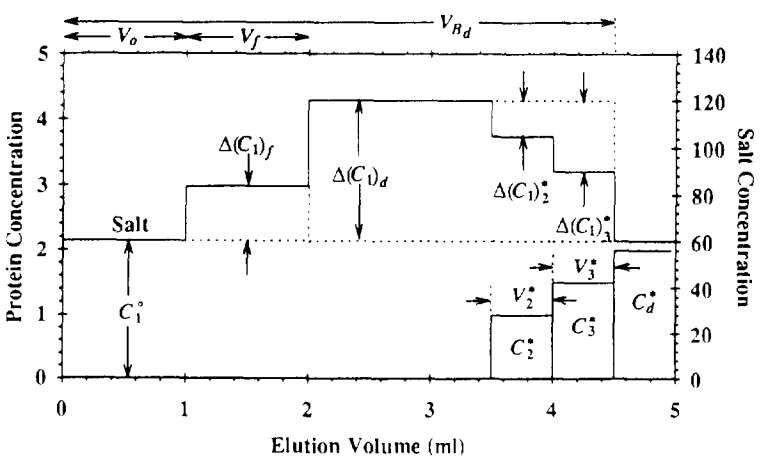

Fig. 1. Ideal SMA ion-exchange displacement separation of a binary feed mixture. From Ref. [9].

Table 1
SMA parameters for proteins and displacers

Protein/ displacer	Characteristic charge (ν)	Steric factor (σ)	σ/ν	Equilibrium constant (K)
α-Chymotryp-sinogen A	4.8	49.2	10.3	$9.2 \cdot 10^{-3}$
Cytochrome *c*	6.0	53.6	8.9	$1.1 \cdot 10^{-2}$
Lysozyme	5.3	34.0	6.4	$1.8 \cdot 10^{-1}$
Protamine	17.5	11	0.6	$1.0 \cdot 10^{9}$
M_r 10 000 DEAE-dextram	21	32	1.5	$6.3 \cdot 10^{-3}$
M_r 40 000 DEAE-dextran	64	130	2.0	$5.5 \cdot 10^{44}$

Chromatographic conditions: SCX column (bed capacity, $\Lambda = 561$ mM), sodium phosphate buffer, pH 6.0.

$$C_i \equiv \frac{\Lambda - \left(C_1^0 + \nu_d C_{fd}\right) \cdot \frac{1}{\lambda}}{-\nu_i \left(\frac{1}{\lambda} - \frac{\Delta}{\Omega}\right)} \tag{8}$$

where the parameters Ω and Λ are defined as:

$$\Omega = \left(\frac{\nu_i}{\nu_i + \sigma_i}\right) \quad \lambda = \sqrt[\nu_i]{\frac{K_{1i}}{\Delta}} \tag{9}$$

The parameters Ω and λ characterize the nonlinear and linear adsorption behavior of the proteins, respectively. The volume (i.e., width) of the isotachic displacement zone, V_i, is determined from a mass balance to be

$$V_i = V_f \cdot \left(\frac{C_{fi}}{C_i}\right) \tag{10}$$

The parameter λ also characterizes the "dynamic affinity" of the proteins under these displacement conditions [11]. Thus, the order of the feed components in the isotachic displacement train is given by

$$\lambda_i < \lambda_j < \ldots < \lambda_n \tag{11}$$

where component i is the first component in the displacement train and component n is the last (note: the order of the feed components in all displacement simulations presented in this manuscript is α-chymotrypsinogen A, cytochrome *c* and lysozyme).

Thus, once the slope of the displacer operating line is determined (Eq. 6), the breakthrough volume can be calculated from Eq. 5. The local salt *microenvironment* for each feed component can then be calculated from Eq. 7. The concentrations and widths of the isotachic zones are determined using Eqs. 8 and 10, respectively. Finally, the order of the feed components are determined from Eq. 11.

3. Results and discussion

The technique described above for calculating isotachic displacement profiles is employed to study the effects of mobile phase salt concentration and displacer equilibrium properties on the concentration of proteins and induced salt gradients in the displacement zones.

3.1. Effect of mobile phase salt concentration

The operating variable which provides the greatest flexibility in the development of ion-exchange displacement separations is the prevailing mobile phase salt concentration. Typically displacement separations are performed with mobile phase salt concentrations in the range of 25 to 150 mM. Within this range, the concentrations and widths of the fully developed displacement zones can vary considerably. In order to examine the effect of this operating variable, displacement separations were simulated for the

separation of a three-component feed mixture (containing the proteins α-chymotrypsinogen A, cytochrome *c* and lysozyme) employing mobile phase salt concentrations of 25, 50 and 75 m*M*. In each of the simulations depicted in Fig. 2, the feed consisted of 1.0 ml of the three feed components at a concentration of 0.75 m*M* each. The simulations correspond to a column with a void volume of 1.0 ml (i.e., $V_0 = 1.0$ ml). The displacer equilibrium parameters were: $K = 6.31 \cdot 10^{-3}$, $\nu = 21$ and $\sigma = 32$. In addition, in order to compare the various separations, the breakthrough volume of the displacer was fixed at 5.5 ml. As seen in the figure, the separation performed at 25 m*M* resulted in the highest concentrations of feed components (note: the order of proteins in all isotachic displacement profiles in this paper was α-chymotrypsinogen A, cytochrome *c* and lysozyme). A major advantage of displacement chromatography is the ability to *concentrate* the feed components while also achieving their separation. Clearly, as seen in the figure, the lower the concentration of salt in the carrier the greater the isotachic concentrations of the feed components.

The separations depicted in Fig. 2 all have the

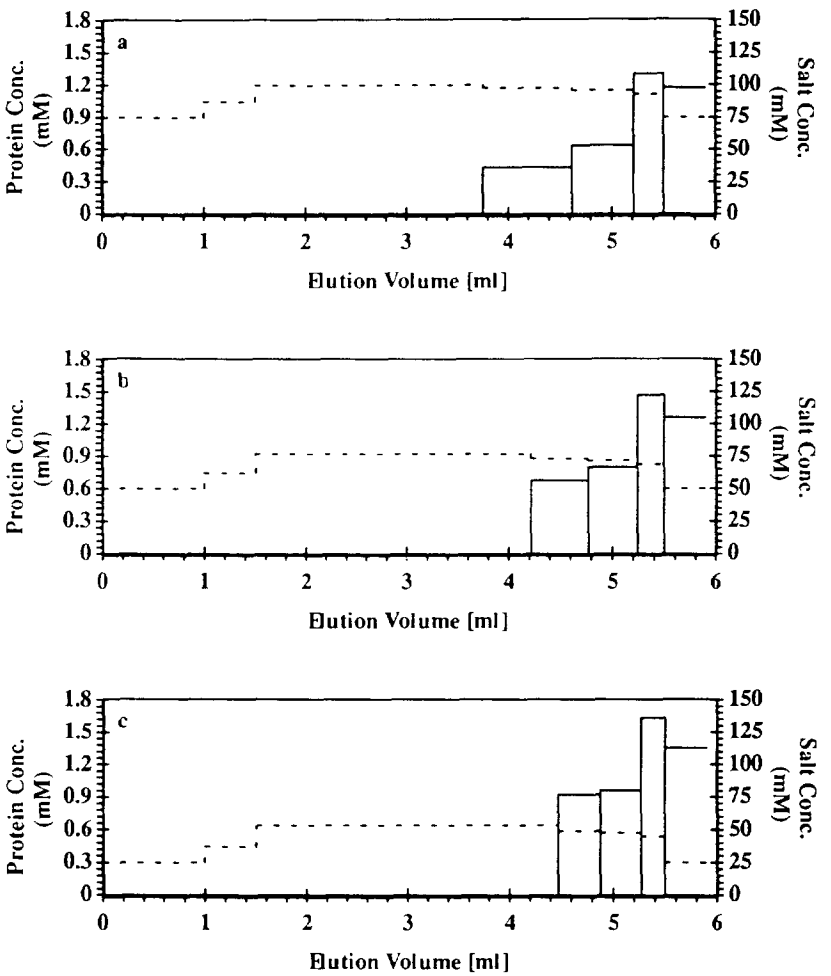

Fig. 2. Effect of mobile phase salt concentration on displacement separations; (a) 75 m*M*; (b) 50 m*M*; (c) 25 m*M*. Displacer: M_r 10 000 DEAE-dextran; feed: α-chymotrypsinogen A, cytochrome *c* and lysozyme, 0.5 ml of 1.0 m*M* each (equilibrium parameters presented in Table 1).

same throughput since the feed amount and separation time are the same. In comparing the separations at 75 and 25 mM, however, it is clear that the 75 mM separation has further approached the maximum feed loading of the column as compared to the 25 mM separation. Thus, the feed mass could be significantly increased in the 25 mM separation; whereas increasing the feed loading at 75 mM would probably result in non-development. In terms of separation performance, this increased feed loading at the lower salt concentration corresponds to greater overall throughput. In general, performing displacement separations at lower mobile phase salt concentrations yields greater throughputs and more concentrated products, each of these a major objective in displacement separations. One *caveat* with respect to this point is that, as the salt microenvironment of the protein bands decrease in magnitude, the kinetics of desorption may adversely affect the separation. It has been observed that the displacement boundaries can become less sharp at extremely low salt concentrations. Furthermore, at very low salt concentrations the elevated protein concentrations may result in precipitation of the purified product. In general, the carrier salt concentration should be decreased until kinetics or precipitation become a problem.

3.2. Effect of displacer equilibrium parameters

Of fundamental importance in displacement chromatography is the design and/or selection of the displacer. It is critical to establish rules of thumb about the effects of displacer SMA parameters on their performance as protein displacers. As defined by Eq. 11, the dynamic affinity of solutes in displacement systems is defined by the magnitude of affinity parameter λ. Thus, the ability of a compound to act as a displacer for a given feed mixture is dictated by this affinity parameter which defines the constraints on the displacer's characteristic charge and equilibrium constant. In this section we will examine the effect of these parameters on the final isotachic displacement profiles. Assuming that the affinity parameter of the displacer is greater than that of any of the feed components, we then have the freedom to employ or design displacers with various combinations of characteristic charge, equilibrium constant and steric factor.

The effect of the displacer's characteristic charge on the final isotachic displacement profile is depicted in Fig. 3. The feed consists of three proteins in 1.0 ml mobile phase at a concentration of 1.0 mM each; the displacer concentration is chosen such that the breakthrough volume is constant at 5.5 ml. The initial mobile phase salt concentration remains constant at 50 mM and the displacer's equilibrium constant and steric factor are $6.31 \cdot 10^{-3}$ and 32, respectively. As seen in the figure, increasing the displacer's characteristic charge results in wider, less concentrated displacement zones. In addition, a higher induced salt gradient results from an increased characteristic charge. In fact, it is the elevated salt concentration in the displaced protein zones, produced by the induced salt gradient, which is responsible for the wider less concentrated zones. Thus, as long as the affinity constraint is satisfied, it may be advantageous to employ a displacer with minimal characteristic charge. This will result in more concentrated displacement zones, higher throughputs, and may also facilitate column regeneration.

The effect of the displacer's steric factor on the final isotachic displacement profile is presented in Fig. 4. The conditions are the same as those in Fig. 3 except that the displacer's steric factor is now allowed to vary from 16 to 64. The equilibrium constant and characteristic charge are constant at $6.31 \cdot 10^{-3}$ and 21, respectively. As seen in the figure, increasing the displacer's steric factor results in narrower, more concentrated displacement zones. Again, the reason for this is the increased induced salt gradient. This is not surprising since an increase in the displacer's steric factor corresponds to a decrease in the overall number of interactions with the stationary phase under non-linear adsorption conditions (thus, less induced salt gradient).

Figs. 3 and 4 indicate that the induced salt gradient can be minimized by increasing the displacer's steric factor and decreasing its characteristic charge. Thus, increasing the ratio of the displacer's steric factor to characteristic charge

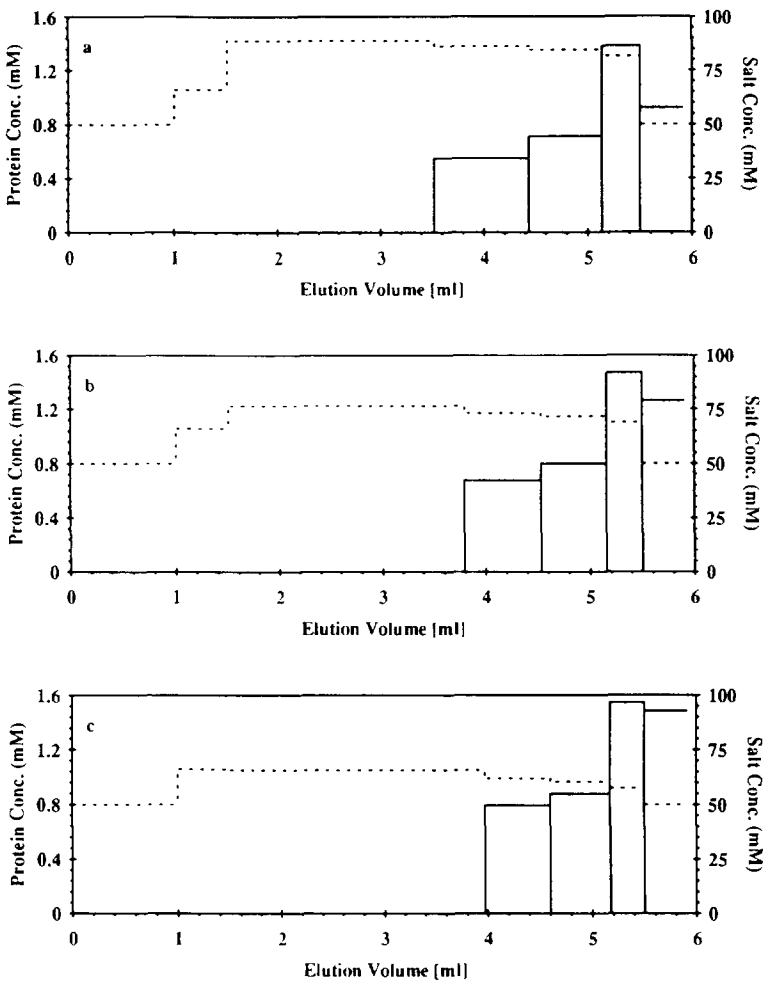

Fig. 3. Effect of displacer characteristic charge on displacement separations; (a) $\nu = 42$; (b) $\nu = 21$; (c) $\nu = 10.5$. Displacer $\sigma = 32$ and $K = 6.31 \cdot 10^{-3}$. (Feed as in Fig. 2).

should increase the performance of the displacement separation. In fact, this ratio has important implications for displacer design. Consider the displacers protamine, M_r 10 000 DEAE-dextran and M_r 40 000 DEAE-dextran; the σ/ν ratios for these cation-exchange displacers are 0.63, 1.52 and 2.03, respectively [6,10]. Thus, from these σ/ν ratios, we would expect the largest induced salt gradient from protamine and the least induced salt gradient from M_r 40 000 DEAE-dextran. Fig. 5 depicts displacement separations employing the σ/ν ratios of these displacers. The feed conditions as well as the displacer breakthrough volumes are the same as in the previous examples. As seen in the figure, the induced salt gradient caused by the protamine front is greater then that of the DEAE-dextran displacers. As already established, this increased induced salt gradient results in broader, less concentrated displacement zones.

In addition to the effect on the final displacement zones, a relatively small value for the σ/ν ratio also restricts the practical operating range of salt in which to perform the displacement separation. The significant induced salt gradient caused by protamine results in a smaller effective window of working salt concentrations in which displacement actually occurs. In fact, operation

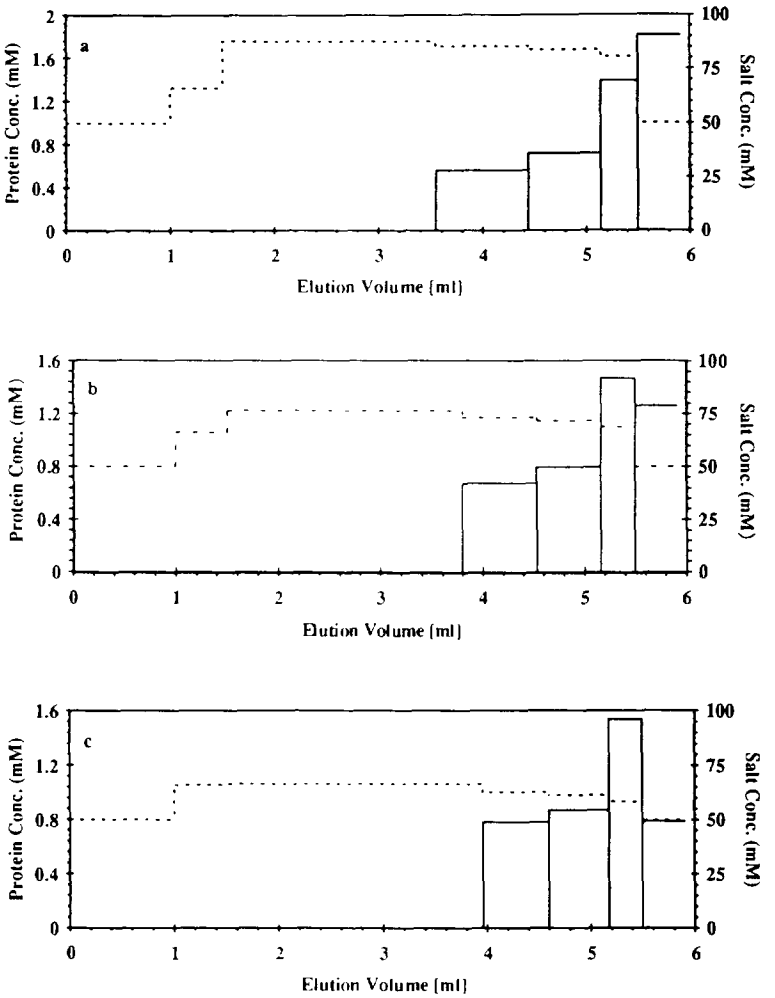

Fig. 4. Effect of displacer steric factor on displacement separations; (a) $\sigma = 16$; b) $\sigma = 32$; c) $\sigma = 64$. Displacer $\nu = 21$ and $K = 6.31 \cdot 10^{-3}$. (Feed as in Fig. 2).

outside of this window substantially depresses the feed component isotherms and can result in the elution of the least retained feed component ahead of the displacement train [6].

4. Conclusions

A significant factor affecting the final isotachic displacement profile is the local salt microenvironment *seen* by the feed components. Clearly, in order to achieve the most concentrated displacement zones, the concentration of salt in the zones must be minimized (allowing for less depression of the feed component isotherms). From the above examples, two effects predominate: the initial carrier salt conditions and the additional induced salt gradient. Thus, it is advantageous to minimize the initial concentration of salt and to choose the displacer which results in the least induced salt gradient (i.e., the highest σ/ν ratio).

It is also important to realize that two displac-

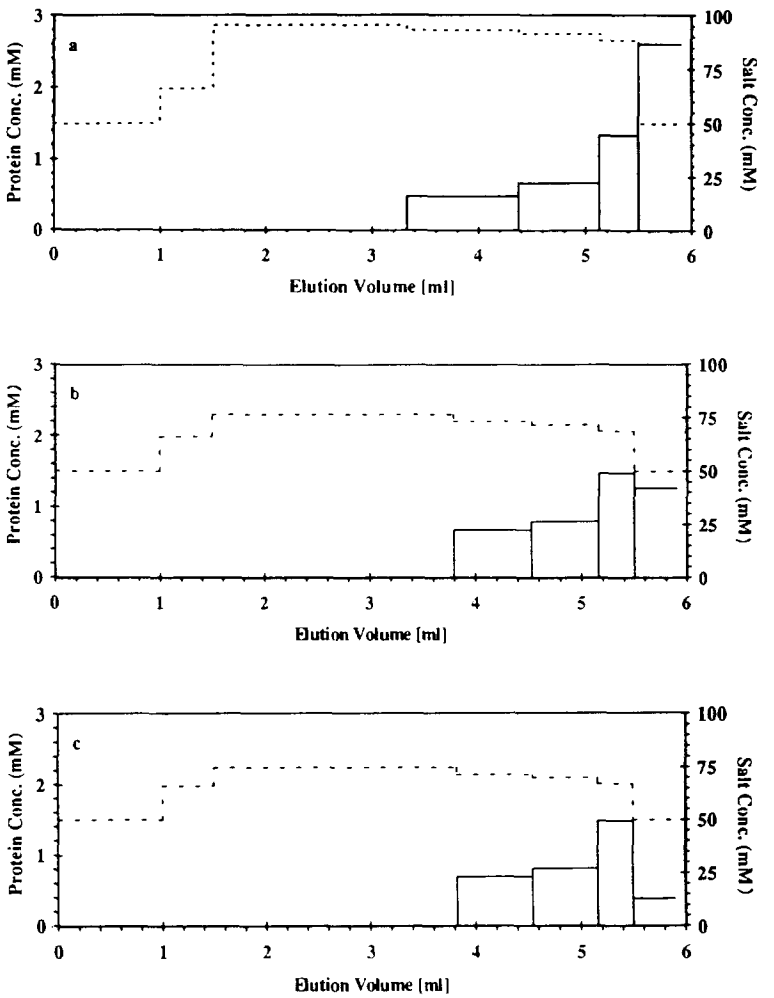

Fig. 5. Effect of displacer steric factor to characteristic charge ratio on displacement separations. (a) Protamine sulfate, $\sigma/\nu = 0.63$; (b) M_r 10 000 DEAE-dextran, $\sigma/\nu = 1.52$; (c) M_r 40 000 DEAE-dextran, $\sigma/\nu = 2.03$. Equilibrium parameters as defined in Table 1. (Feed as in Fig. 2).

ers with significantly different characteristic charges can effect the same induced salt gradient if the σ/ν ratios and the breakthrough volumes are identical. Thus, a highly charged high affinity polymer does not necessarily act as a better displacer than a low-molecular-mass displacer, providing it has sufficient affinity [12]. A detailed analysis of the effect of displacer properties on the transient behavior of ion-exchange displacement chromatography is currently being carried out using both rigorous computer simulations and experiments and will be the subject of a future report.

Symbols

C	mobile phase concentration, mM
$\Delta(C_1)$	change in salt concentration due to a front, mM
K	equilibrium constant, dimensionless

Q stationary phase concentration (per volume SP), mM
V volume, ml

Subscripts and Superscripts

$^{-}$ sterically non-hindered
* unit selectivity
^{0} initial (*i.e.*, carrier)
$_{0}$ unretained solute
$_{1}$ salt
$_{a}$ solute a
$_{B}$ breakthrough
$_{d}$ displacer
$_{f}$ feed
$_{i}$ solute i
$_{n}$ solute n

Greek letters

β column phase ratio, $(1-\varepsilon_t)/\varepsilon_t$, dimensionless
Δ slope of displacer operating line, dimensionless
ε_t total porosity (*i.e.*, inter- and inter-particular), dimensionless
λ isotachic affinity parameter (Eq. 9), dimensionless
Λ stationary phase capacity (monovalent salt counterions), mM
ν characteristic change, dimensionless
σ steric factor, dimensionless
Ω adsorption parameter (Eq. 9), dimensionless

References

[1] E.A Peterson, *Anal. Biochem.*, 90 (1978) 767.
[2] E.A Peterson and A.R. Torres, *Anal. Biochem.*, 130 (1983) 271.
[3] A.W. Liao, Z. El Rassi, D.M. LeMaster and Cs. Horváth, *Chromatographia*, 24 (1987) 881.
[4] G Subramanian and S.M. Cramer, *Biotechnol. Prog.*, 5 (1989) 92.
[5] S.C.D. Jen and N.G. Pinto, *J. Chromatogr. Sci.*, 29 (1991) 478.
[6] J.A. Gerstner and S.M. Cramer, *Biotechnol. Prog.*, 8 (1992) 540.
[7] J.A. Gerstner and S.M. Cramer, *BioPharm.*, 5 (1992) 42.
[8] G. Jayaraman, S.D. Gadam and S.M. Cramer, *J. Chromatogr.*, 630 (1993) 53.
[9] C.A. Brooks and S.M. Cramer, *AIChE J.*, 38 (1992) 1969.
[10] S.D. Gadam, G. Jayaraman and S.M. Cramer, *J. Chromatogr.*, 630 (1993) 37.
[11] C.A. Brooks and S.M. Cramer, *Chem. Eng. Sci.*, (1994) in press.
[12] G. Jayaraman, Y.F. Li, J.A. Moore and S. M. Cramer, *J. Chromatogr. A*, in press.